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Investigation of α_1-adrenoceptor subtypes mediating vasoconstriction in rabbit cutaneous resistance arteries

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1 Cutaneous resistance arteries (c.r.a.) (internal diameter = 240.94 ± 5.42 μm, n = 67/25 (number arteries/number animals)) from New Zealand white rabbits were mounted in wire myographs and a normalization procedure followed.

2 Cumulative concentration-response curves (CCRCs) were constructed for the α-adrenoceptor agonists noradrenaline (NA), (R)A61603 and phenylephrine (PE) in the presence of cocaine (3 μM), propranolol (1 μM) and corticosterone (10 μM). The effects of competitive α_1-adrenoceptor antagonists, prazosin, WB4101, 5-methyl-urapidil, HV723, BMY7378 and the irreversible α_{1B} selective compound chloroethylclonidine (CEC) were examined versus the potency and maximum response of the c.r.a.s to noradrenaline.

3 The high potency of A-61603 relative to PE has been shown to differentiate both functional and binding site α_{1A}- or α_{1B}-adrenoceptors from α_{1D}-adrenoceptors: A-61603 was 944 times more potent than phenylephrine (at EC_{50}) suggesting the presence of a functional α_{1A} or α_{1B} as opposed to an α_{1D}-subtype.

4 Exposure to chloroethylclonidine (CEC; 100 μM) decreased the maximum response to noradrenaline but did not significantly change noradrenaline sensitivity indicating that a substantial part of noradrenaline-induced vasoconstriction in rabbit cutaneous arteries is CEC-insensitive.

5 The potencies of prazosin (pA_2 = 9.14) and WB4101 (pA_2 = 9.30) indicate the involvement of prazosin-sensitive functional α_1-adrenoceptors. The slopes of corresponding Schild plots for prazosin and WB4101 did not include negative unity which implies the possible involvement of more than one functional α_1-adrenoceptor subtype in noradrenaline-induced vasoconstriction in rabbit cutaneous resistance arteries. In contrast to this, in the case of 5-methyl-urapidil and HV723, the Schild plot slope parameters were not significantly different from negative unity over the range of concentrations used; the low pA_2 value for 5-methylurapidil (7.27) suggests the non-involvement of an α_{1A}- or an α_{1D}-adrenoceptor; the low pA_2 value for HV723 (8.47) was similar to that against responses postulated as α_{1L}.

6 We conclude that rabbit cutaneous resistance arteries express a prazosin-sensitive functional α_1-adrenoceptor resembling the α_{1B} and another low affinity site for prazosin which on the basis of the functional antagonism produced by HV723 most closely resembles the α_{1L}-adrenoceptor; the low pA2 value for HV723 (8.47) is similar to that against responses postulated as α_{1L}.

Keywords: Cutaneous resistance arteries; α_1-adrenoceptor subtypes

Introduction

α_1-Adrenoceptors are a heterogeneous group of receptors the subclassification of which is far from resolved. It has long been known that the agonist potency series at α_1-adrenoceptors varies greatly between tissues (Ruffolo, 1985). The first attempt at subclassifying α_1-adrenoceptors into α_{1a}- and α_{1b}-adrenoceptors was partly based on the variable potency series for agonists in different tissues (McGrath, 1982) and, recently, it has been demonstrated that the relative potencies of two agonists, phenylephrine and A-61603 can distinguish between subtypes which have been defined by other means (Knepper *et al*., 1995). Later subclassification schemes were mainly based on radioligand binding studies, initially of native receptors (Battaglia *et al*., 1983; Morrow & Creese, 1986) and later of recombinant receptors (Schwinn & Lomasney, 1992; Forray *et al*., 1994); there is relatively less information on the subclassification of functional α_1-adrenoceptors; the present classification scheme of these receptors remains dominated by sensitivity to prazosin.

Although there is a wide continuum of prazosin potency (Drew, 1985) amongst functional α_1-adrenoceptors, it has been suggested that they should be subdivided into prazosin-sensitive (high: α_{1H}) and prazosin-insensitive (low: α_{1L}) subtypes (Flavahan & Vanhoutte, 1986). Subsequently, when α_1-adrenoceptors were cloned, the 3 clones, when re-expressed, all showed high sensitivity to prazosin, so that the functional equivalents of those 3 clones termed α_{1a}, α_{1b} and α_{1d} (Bylund *et al*., 1994) should all be subsets of the α_{1H} (Muramatsu *et al*., 1991).

Prazosin-sensitive functional α_1-adrenoceptors have been divided into α_{1A} and α_{1B} on the basis of WB4101 affinity in ligand binding (Morrow & Creese, 1986); this can be translated to the functional level, for example, WB4101 can distinguish these subtypes by blocking noradrenaline-induced contraction in rat vas deferens (α_{1A}, K_B = 0.3 nM) with higher affinity than in rat spleen (α_{1B}, K_B = 5.4 nM) (Han *et al*., 1987). From this original definition with two subtypes, α_{1A} and α_{1B}, the α_{1A} has now been divided, for a number of reasons, into α_{1A} and α_{1D} (Bylund *et al*., 1994): at the level of functional competitive antagonism this separation is not easy to make, although recently BMY7378 (Goetz *et al*., 1995) has been postulated as selective for α_{1D} over α_{1A}.

Some, but not all, 'prazosin-insensitive' α_{1L}-adrenoceptors have relatively low affinity for HV723 (K_B = 2–7 nM) compared with the prazosin-sensitive sites which have relatively higher affinity for this compound (K_B = 0.4–1 nM); this low affinity for HV723 in conjunction with low affinity for prazosin is thus a further marker for the α_{1L}-adrenoceptor (Muramatsu *et al*., 1990b).

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Despite this complex background, it is important to attempt to establish which α_1-adrenoceptor subtypes are involved in the control of vascular resistance (McGrath *et al*., 1996). For example, currently, there is a controversy over whether α_1-subtype selectivity will convey selectivity on α_1-adrenoceptor antagonists in relieving the symptoms of benign prostatic hypertrophy while avoiding hypotensive side effects (Forray *et al*., 1994). It has long been known that both α_1- and α_2-adrenoceptors produce vasoconstriction in several species, including rat, rabbit and dog (Docherty & McGrath, 1980; McGrath *et al*., 1982; Alabaster *et al*., 1985; Guimaraes & Nunes, 1990). However, few *in vitro* studies have attempted to classify whether the α_1- or α_2-adrenoceptors are involved in mediating vasoconstriction in resistance arteries; some studies have suggested that responses to exogenously applied and neurally released noradrenaline in human subcutaneous resistance arteries are mediated by both α_1- and α_2-adrenoceptors (Nielsen *et al*., 1990; Parkinson *et al*., 1992).

Other studies have suggested that vasoconstriction of arterioles and venules is mediated by different α-adrenoceptor subtypes (Leech & Faber, 1996); vasoconstriction in rat skeletal muscle arterioles being mediated predominantly by an α_{1D}-like receptor and an α_{2D}- whereas constriction of venules is dominated by the α_{1B}- and the α_{2D}-adrenoceptor subtypes. Van der Graaf *et al*. have attempted to subclassify the α_1-adrenoceptors which mediate vasoconstriction in both rat aorta (Van der Graaf *et al*., 1996a) and rat mesenteric resistance vessels (Van der Graaf *et al*., 1996b); their analysis concluded that there was more than one subtype of functional α_1-adrenoceptor present in the aorta and that α_{1L}-adrenoceptors mediated part of the noradrenaline-induced vasoconstriction observed in rat small mesenteric arteries. We have published data which indicate that there are developmental changes in functional α_1-adrenoceptor subtypes in rat mesenteric resistance arteries (Smith & McGrath, 1996). These studies indicate that there may be an α-adrenoceptor subtype-specific role in the regulation of vascular tone, which could be altered with age and cardiovascular disease and is therefore of relevance to the use of adrenoceptor ligands in treatment of cardiovascular diseases or other pathologies involving tissues modulated by catecholamines.

The aim of this study was to investigate, *in vitro*, the α_1-adrenoceptors subtype(s) mediating vasoconstriction of rabbit cutaneous resistance arteries, which preliminary studies had shown to contract to agonists in a potency series consistent with α_1- but not α_2-adrenoceptors, i.e., phenylephrine $>>$ UK14304 (Macmillan *et al*., 1994). To this end we investigated the potency series for the agonists, noradrenaline, phenylephrine and (**R**)A-61603 and the antagonist potency of the key antagonists prazosin, WB4101, 5-methyl-urapidil, HV723 and BMY7378.

Some of these results have been published in abstract form (Smith *et al*., 1996).

Methods

Rabbit cutaneous artery preparation

Experiments were carried out in male New Zealand white rabbits weighing 3.0–3.5 kg. They were killed by an overdose of pentobarbitone into the ear vein and a flap of skin from the area overlying the gluteal muscles was removed. Connective tissue was cleared from above the network of cutaneous arteries and resistance arteries (2 mm length) were isolated and excised under a dissecting microscope.

Cutaneous resistance arteries (internal diameter = 240.94 ± 5.42 μm, $n = 67/25$) were mounted as ring preparations in a Mulvany-Halpern double myograph (J, P Trading, Aarhus, Denmark), mounted on two 40 μm steel wires which are attached to a force transducer and a micrometer, as described by Mulvany & Halpern (1977). The vessel was bathed in Krebs-Henseleit solution (in mM: NaCl 118.4, KCl 4.7, $MgSO_4.H_20$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.9, $CaCl_2$ 2.5, glucose 11.1, EDTA 0.023), kept at 37°C and gassed with 95%O_2/5%CO_2 mixture. Additionally, cocaine (3 μM), propranolol (1 μM) and corticosterone (10 μM) were present, in all experiments.

Normalization procedure

After a rest period of 30 min, the artery was stretched at 1 min intervals to determine the exponential passive wall tension-internal circumference (L) relationship. From the Laplace relationship, where $P = T/r$ (P is the effective pressure, T is wall tension and r is the internal radius), the circumference (L_{100}) was calculated by an iterative computer method that gave an equivalent transmural pressure difference at 100 mmHg for each artery. The circumference at $0.9 \times L_{100}$ was calculated where the active force production was close to maximum (data not shown). Normalized vessel internal diameter for the remainder of the experiment was set at $0.9 \times L_{100}$. From the known length-tension relationship we calculated the equivalent wall tension at $0.9 \times L_{100}$ and the equivalent effective pressure P_1 (mmHg). Effective pressure is an estimate of the pressure which would be necessary to extend the vessel to the measured internal circumference. If the arteries had a normalized internal diameter greater than 300 μm, they were excluded from our study.

α_1-Adrenoceptor agonists

After the normalization procedure, the arteries were exposed to noradrenaline (NA, 10 μM), until equilibrium contraction was established and then washed. Thirty minutes later, a cumulative dose-response curve at half log unit steps was generated to noradrenaline (1–1000 nM), phenylephrine (1–10,000 nM) or (**R**)A-61603 (0.01–1000 nM) in half log unit concentration increments, in the presence of cocaine (3 μM), propranolol (1 μM) and corticosterone (10 μM). The CRC to noradrenaline was repeated and data derived from the second curve since time-control studies showed that the CRC to NA varied between the first and second CRCs but was consistent in the subsequent CRCs thereafter, up to the fifth (data not shown).

α_1-Adrenoceptor antagonists

The procedure followed was essentially the same as in the α_1-agonist protocol for NA. Five CRCs to NA were constructed. The second curve served as the control and three increasing concentrations of test antagonists were added 30 min before the third to fifth CRCs: the α_1-adrenoceptor antagonists used were prazosin, WB4101, HV723, 5-methyl-urapidil and BMY7378. Antagonist potency was expressed as a pA_2 value which was obtained from the x-intercept of the plot of log (agonist DR − 1) against log (antagonist concentration) (Arunlakshana & Schild, 1959).

Chloroethylclonidine

After an initial exposure to NA (10 μM), 2 CRCs were constructed to NA; the second of which served as a control; followed by exposure to chloroethylclonidine (10 μM or 100 μM) for 60 min, 30 min of washing with Krebs (10× washes) and a second CRC to NA (O'Rourke *et al*., 1995; Williams & Clarke, 1995).

Statistics

Contraction responses were expressed as increase in active effective pressure (P, mmHg), calculated as increase in isometric tension (T) above resting divided by the normalized internal radius. Responses were averaged at each concentration of the agonist. Agonist potency was expressed in terms of a pD_2 value which represents the negative log of the concentration of the

agonist required to produce 50% of the maximum response. Antagonist pA_2 values and slopes of Schild regressions were calculated by use of GraphPad Prism Version 2.0 (GraphPad Software Inc., San Diego, CA). Differences were considered significant at a level of $P<0.05$.

Drugs

The following drugs were used: chloroethylclonidine dihydrochloride (CEC; Research Biochemicals Incorporated (RBI)), cocaine HCl (Macarthys), corticosterone 21-acetate (Sigma), HV723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxy phenoxyethyl)-amino)-propyl)benzeneacetonitrile fumarate) (gift I. Muramatsu), (−)-noradrenaline bitartrate (Sigma), phenylephrine hydrochloride (Sigma), prazosin hydrochloride (Sigma), propanolol HCl (Sigma), WB4101 (2-(2.6-dimethoxyphenoxyethyl)-aminomethyl-1-benzodioxane) (Research Biochemicals Incorporated), 5-methyl-urapidil (Research Biochemicals Incorporated), (**R**)A-61603 (N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]-methanesulfonamide hydrobromide) (gift; from Dr Michael Meyer, Abbott laboratories).

All concentrations of drugs are expressed as a final concentration in the myograph. All drugs were prepared from salts each day in deionized water with the exception of noradrenaline, which was dissolved in 23 μM Na_2 EDTA, and corticosterone, which was dissolved in ethanol.

Results

Noradrenaline produced a concentration-response curve with a pD_2 of 7.06±0.08 (n=53/25) (n=vessels/rabbits) (mean±s.e.mean, −log M) and a maximum contraction of 36.42±2.32 mN mm^{-2}. Phenylephrine and (**R**)A-61603 also produced concentration-response curves in the rabbit cutaneous arteries with pD_2 and maximum contraction values of: phenylephrine 6.13±0.17, 39.55±3.18 mN mm^{-2}.(n=16/8); (**R**)A-61603 9.11±0.07, 41.29±3.12 mN mm^{-2} (n=16/8) (Figure 1 and Table 1).

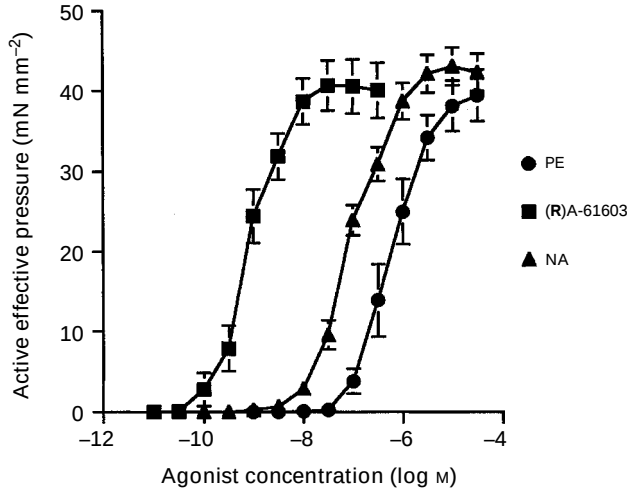

Figure 1 Cumulative concentration-responses curves to phenylephrine (PE; n=16/8), (**R**)A-61603 (n=16/8) and noradrenaline (NA; n=53/25) in rabbit cutaneous resistance arteries. Vertical lines show s.e.mean.

Prazosin, WB4101, HV723 and 5-methyl-urapidil produced concentration-dependent shifts in the potency of NA without reducing the maximum response over the ranges used for analysis. 5-Methyl-urapidil (100 μM) depressed the maximum and virtually abolished the response and so was not included. Schild plots were constructed from the effects of a range of concentrations of each antagonist and pA_2 values were calculated (Figures 2(a and b), 3(a and b), 4(a and b) and 5(a and b) and Table 2). BMY7378 was tested across the range 0.01, 0.1 and 1 μM in order to cover the range of potency against α_{1D}-adrenoceptors, but this proved too low to construct a Schild plot. The estimated pK_B for BMY7378 with shifts at 0.1 and 1 μM was 7.06±0.21 (n=7/4).

Exposure to the irreversible antagonist CEC (100 μM) decreased the maximum response to NA but did not result in a significant change in noradrenaline sensitivity (NA pD_2 pre-CEC=7.02±0.19 (n=8/4); NA pD_2 post-CEC=6.40±0.57 (n=8/4) (Figure 6). CEC (10 μM) had no effect on either the maximum response or the sensitivity to noradrenaline.

Discussion

In this study, we have examined, for the first time, the subtypes of functional α_1-adrenoceptors mediating vasoconstriction in rabbit cutaneous resistance arteries, by use of both selective agonists and antagonists. Previous studies have shown that α_1-adrenoceptors play a role, along with α_2-adrenoceptors in mediating vasoconstriction in 'resistance' arteries from both man and rats (Nielsen *et al*., 1990; Stephens *et al*., 1992; Leech & Faber, 1996).

The main hindrance in the subclassification of functional α_1-adrenoceptors is the lack of compounds which not only can distinguish different subtypes of α_1-adrenoceptor binding sites but also can reproduce their subtype-selectivity at functional α_1-adrenoceptors. The agonist potency of A-61603 relative to PE has been shown to differentiate functional α_{1A} and α_{1B} from α_{1D}-adrenoceptors (Knepper *et al*., 1995): A-61603 is a potent agonist at α_{1A}-adrenoceptors in rat vas deferens (200 to 300 fold more potent than NA or PE, respectively) and in canine isolated prostate strips (130 to 165 fold more potent than NA or PE, respectively). In contrast to this, A-61603 is only 40 fold more potent than PE at α_{1B} sites in rat spleen and is 35 fold less potent at rat aortic α_{1D} sites.

According to this hypothesis, the relative potency of (**R**)A-61603 to PE in rabbit cutaneous resistance arteries (see Table 1) indicates the involvement of the α_{1A}- or α_{1B}-adrenoceptor subtypes in vasoconstriction, as opposed to the α_{1D} subtypes, although the resulting, very high, PE/(**R**)A61603 potency ratio is outwith the range for even the α_{1A}-subtype. This may be due to our use of the more potent (**R**) enantiomer as opposed to the

Table 1 Agonist potencies

	Cutaneous resistance artery EC_{50}	*'α_{1A}' rat vas deferens* EC_{50}	*'α_{1A}' canine prostate* EC_{50}	*'α_{1B}' rat spleen* EC_{50}	*'α_{1D}' rat aorta* EC_{50}
Noradrenaline	87	1230	2590	10800	12.8
A-61603	*0.78	6.16	20.1	380	6550
Phenylephrine	736	2050	3330	15700	198
NA/A-61603 ratio	112	200	129	28	0.002
PE/A-61603 ratio	944	333	166	41	0.03

The values represent the potencies (EC_{50} × 1nM) of A-61603, phenylephrine (PE) and noradrenaline (NA) in cutaneous resistance arteries compared with their potencies in other functional studies (reproduced from Knepper *et al*., 1995). *Represents the *R* enantiomer of A-61603.

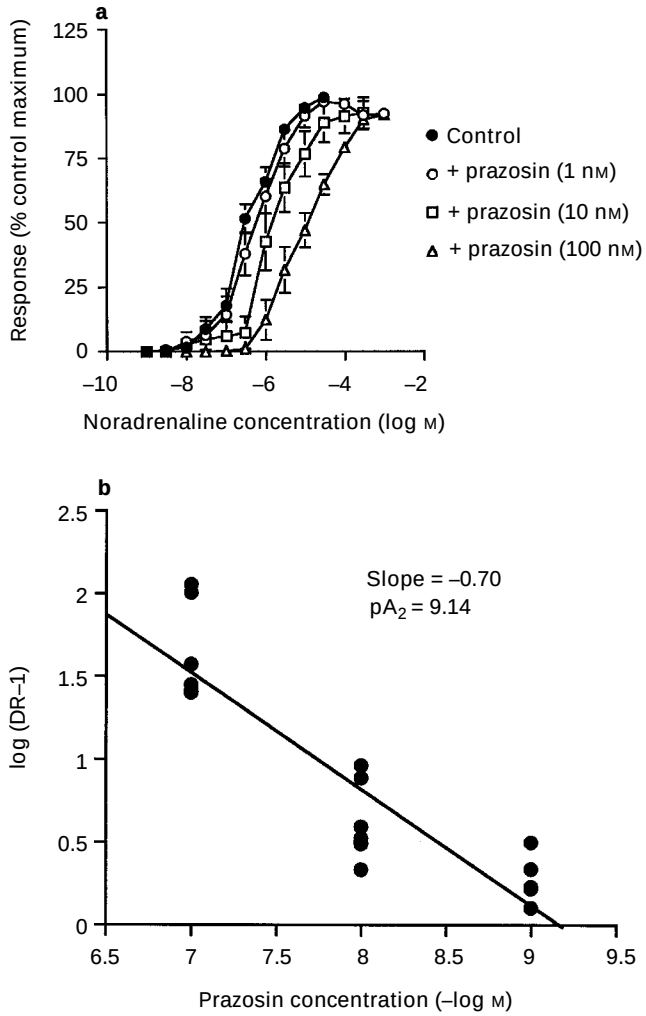

Figure 2 (a) Concentration-response curves to noradrenaline in the absence and presence of prazosin (1, 10 and 100 nM) and (b) pA_2 values obtained for the competitive antagonist prazosin. Each point represents an individual experiment. (Full details of pA_2 values are shown in Table 2).

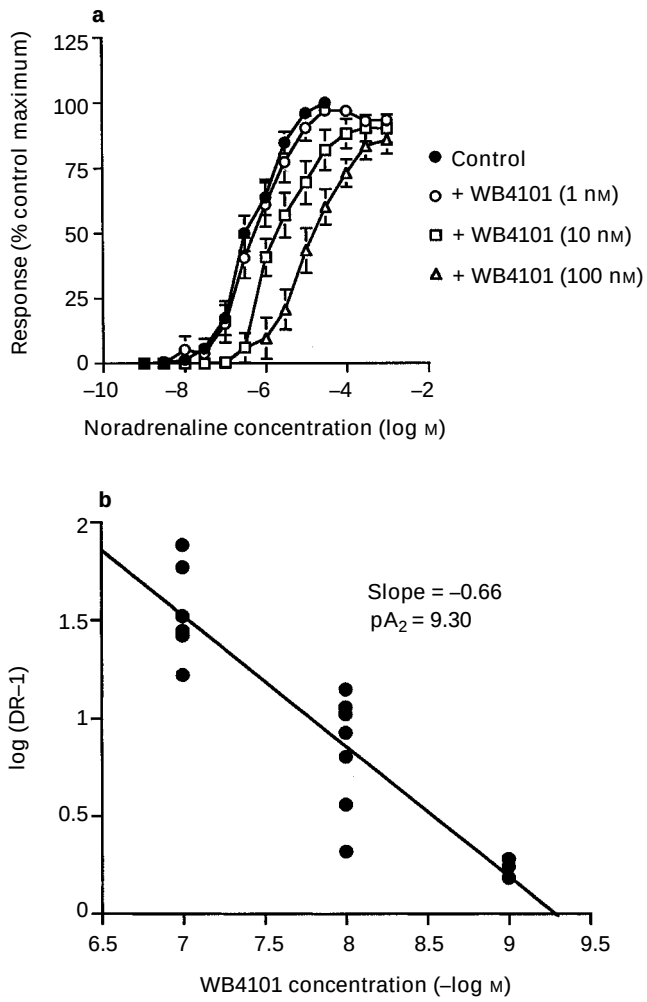

Figure 3 (a) Concentration-response curves to noradrenaline in the absence and presence of WB4101 (1, 10 and 100 nM) and (b) pA_2 values obtained for the competitive antagonist WB4101. Each point represents an individual experiment. (Full details of pA_2 values are shown in Table 2).

racemic compound. It should also be noted that all three agonists were very much more potent on rabbit c.r.a. than on the tissues used as examples of α_{1A}- and α_{1B}-subtype. We know of no data on the effects of (**R**)-A61603 at examples of α_{1L}. The high potency of (**R**)A-61603 may be due to it having a high potency at the α_{1L}-adrenoceptors; previously some authors have suggested that α_{1L}-adrenoceptors mediate noradrenaline-induced vasoconstriction in rat vas deferens (Muramatsu *et al.*, 1995). If it is concluded from the present study that α_{1L}-adrenoceptors are involved in rabbit c.r.a. then these receptors also may be sensitive to this agonist.

Contractions to NA were potently inhibited by prazosin, WB4101, HV723 and 5-methyl-urapidil, without significantly affecting the maximum response to NA. Analysis of the Schild plots for prazosin and WB4101 indicated that the resulting slopes did not include negative unity, their values suggesting the possible involvement of more than one α_1-adrenoceptor type (Kenakin, 1982). This is reflected in the non-parallel shift in the lowest part of the CRC, evident in Figures 2(a) and 3(a).

The potencies of prazosin (prazosin 1 nM; $pK_B = 9.28 \pm 0.07$) and WB4101 (WB4101 1 nM: $pK_B = 9.23 \pm 0.03$) suggest the presence of a 'high affinity for prazosin' α_1-adrenoceptor subtype; the high affinity for WB4101 might suggest an α_{1A}-subtype. However, the resulting low slopes from their Schild plots suggest the involvement of an additional low prazosin-affinity α_1-subtype. Evidence for this is provided by the estimated pK_B values derived for the higher concentrations of prazosin and WB4101, which point to lower affinities than either the pK_B estimated from the lowest antagonist concentrations or the extrapolated pA_2 values, which take all antagonist concentrations into account. This focuses attention on the effects of the lowest concentrations of prazosin and WB4101, which, essentially, were more effective than would be expected from the effects of higher concentrations and suggest that, at low concentrations (1 nM), these two antagonists may be identifying a receptor for which they have high affinity and which accounts for the response to the lowest concentrations of noradrenaline. A corollary of this is that the response which remains to NA in the presence of low concentrations of prazosin and WB4101 is more resistant to blockade, i.e. has lower affinity for prazosin and WB4101, suggesting that NA is acting through this receptor at a higher concentration range. Taking all this into account, the low absolute potency of HV723 ($pA_2 = 8.47$) relative to prazosin and WB4101 supports the presence of a functional α_{1L}-adrenoceptor, as defined by Muramatsu *et al.* (1990b).

The low potency of 5-methyl-urapidil ($pA_2 = 7.27$) and the substantial shift in NA maximum response produced by pre-exposure to chloroethylclonidine (Figure 6) argue against the classification of the high prazosin-affinity site as an α_{1A} subtype.

The Schild plots, close to unity, of 5-methyl-urapidil and HV723 suggest that neither of these compounds distinguishes

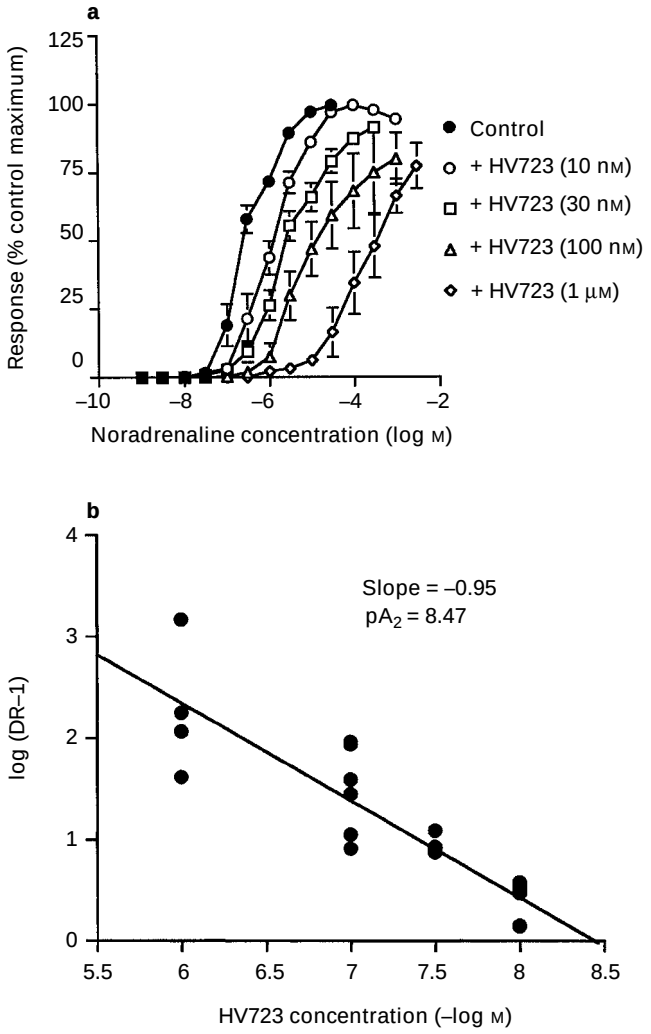

Figure 4 (a) Concentration-response curves to noradrenaline in the absence and presence of HV723 (10, 30 and 100 nM) and (b) pA_2 values obtained for the competitive antagonist HV723. Each point represents an individual experiment. (Full details of pA_2 values are shown in Table 2).

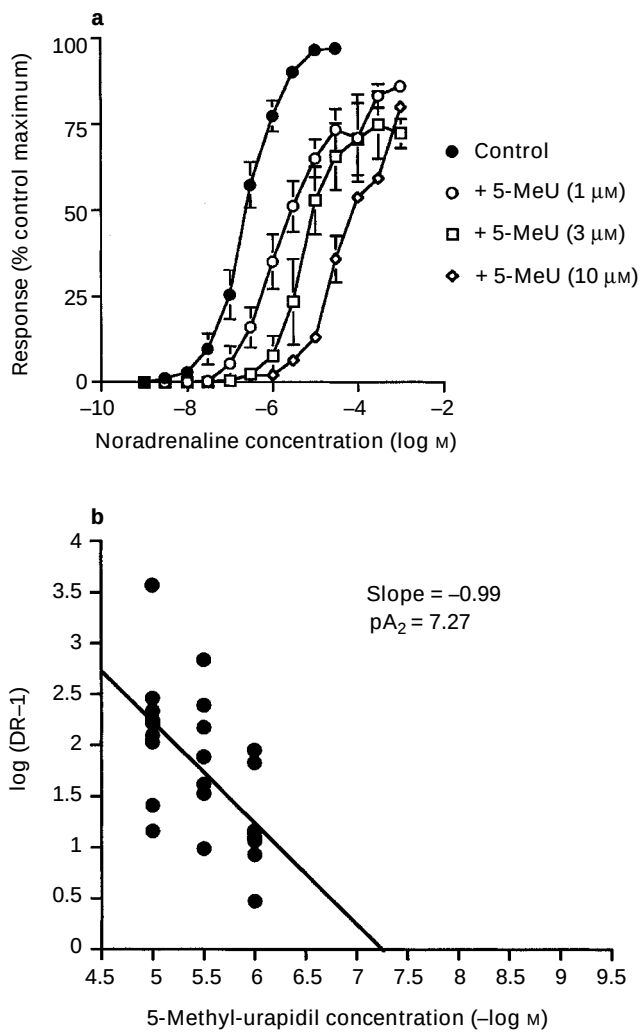

Figure 5 (a) Concentration-response curves to noradrenaline in the absence and presence of 5-methyl-urapidil (5-MeU; 1, 30 and 100 μM) and (b) pA_2 values obtained for the competitive antagonist 5-methyl-urapidil. Each point represents an individual experiment. (Full details of pA_2 values are shown in Table 2).

Table 2 Potencies of antagonists against contractions to noradrenaline (NA) in rabbit cutaneous resistance arteries and slopes from corresponding Schild plots

Antagonist	*pA_2*	*Slope*	*log[Antagonist]*	*pK_B*
Prazosin	9.14	−0.70	−9	9.28±0.07 (n=5)
		(−0.90 to −0.50)	−8	8.61±0.09 (n=7)
			−7	8.65±0.12 (n=6)
WB4101	9.30	−0.66	−9	9.23±0.03 (n=3)
		(−0.84 to −0.48)	−8	8.83±0.11 (n=7)
			−7	8.54±0.10 (n=6)
HV723	8.47	−0.95	−8	8.37±0.09 (n=5)
		(−1.21 to −0.70)	−7.5	8.47±0.04 (n=5)
			−7	8.57±0.24 (n=6)
			−6	8.28±0.33 (n=4)
5-Methyl-urapidil	7.27	−0.99	−6	7.19±0.13 (n=10)
		(−1.53 to −0.44)	−5.5	7.30±0.17 (n=7)
			−5	7.05±0.16 (n=9)
BMY 7378			−7	7.42±0.28 (n=3)
			−6	6.72±0.28 (n=4)

Potencies are expressed as pA_2 values from the Schild plots shown in Figures 2–5 and pK_B values±s.e.mean, which were calculated from the shift in noradrenaline potency produced by individual antagonist concentrations. Slopes from the respective Schild plots (±95% confidence limits) are presented.

two subtypes. From the literature, in studies of functional α_1-adrenoceptor subtypes, 5-methyl-urapidil is unable to distinguish between the α_{1B}- and the α_{1L}-subtypes, having similar potencies at both the human internal iliac artery (α_{1B}-) ($pA_2 = 7.43 \pm 0.22$; Hatano *et al*., 1994) and the rabbit prostate (α_{1L}-) ($pA_2 = 7.87 \pm 0.08$; Hiraoka *et al*., 1995). It is also evident from the literature that HV723 has similar affinities at both the α_{1L}- of rabbit prostate ($pK_i = 8.36 \pm 0.07$; Hiraoka *et al*., 1995) and the α_{1B}-subtype of rat liver ($pK_i = 8.88 \pm 0.05$; Ohmura & Muramatsu, 1996). Hence, their Schild slopes, which include negative unity, implying competitive antagonism at a single receptor site, are also consistent with two sites at which they have similar affinity, reflecting the inability of 5-methyl-urapidil or HV723 to distinguish between α_{1B}- and α_{1L}-adrenoceptor subtypes. If their absolute values for pA_2s are compared with the literature, then both appear low, which would also indicate α_{1B}-receptors identified by 5-methyl-urapidil and α_{1L}-receptors identified by HV723.

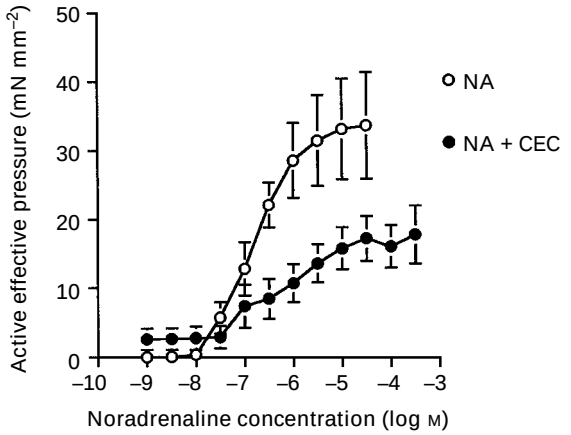

Figure 6 Effects of chloroethylclonidine (CEC, 100 μM) on the contractions to noradrenaline (NA) in rabbit cutaneous resistance arteries in the presence of cocaine, propranolol and corticosterone ($n = 8/4$).

The pA_2 for 5-methyl-urapidil in rabbit cutaneous arteries in our study (7.27) is similar to the pK_B which Leech and Faber (1996) obtained for the same drug in rat (cremaster) skeletal muscle arterioles (7.35 ± 0.11). Our pA_2 for WB4101 is a little higher (9.26) than the pK_B estimated by Leech and Faber (8.82 ± 0.14). However, they derived affinity from single concentrations rather than a range of concentrations of antagonists. With WB4101 10 nM, the concentration used by Leech & Faber, we obtained an identical pK_B to theirs (8.83 ± 0.11). The affinity of the α_{1D}-selective compound, BMY7378 is also similar between the two studies; its apparent dissociation constant (pK_B) at rat skeletal arterioles was 6.86 ± 0.25, which is similar to its affinity in our study of rabbit cutaneous resistance arteries ($pK_B = 7.06 \pm 0.21$). Leech and Faber concluded that rat skeletal arterioles contain the α_{1D}-adrenoceptors, based on the supposition that the relatively high affinity of WB4101 was more important than the very low apparent affinity of BMY7378, which would be expected to have an affinity of $\sim$1 nM at functional α_{1D}-adrenoceptors (Piascik *et al*., 1995). In the light of this, the low affinity of BMY7378 from our study, supported by the agonist series, argues against the involvement of α_{1D}-adrenoceptors in noradrenaline-induced vasoconstriction in rabbit cutaneous resistance arteries; perhaps the rat cremaster arterioles are similar.

The reduction of the noradrenaline maximum by chloroethylclonidine (100 μM), without any shift in sensitivity, could be taken as evidence of two receptor subtypes, one sensitive and one insensitive to this compound, presumably the sensitive

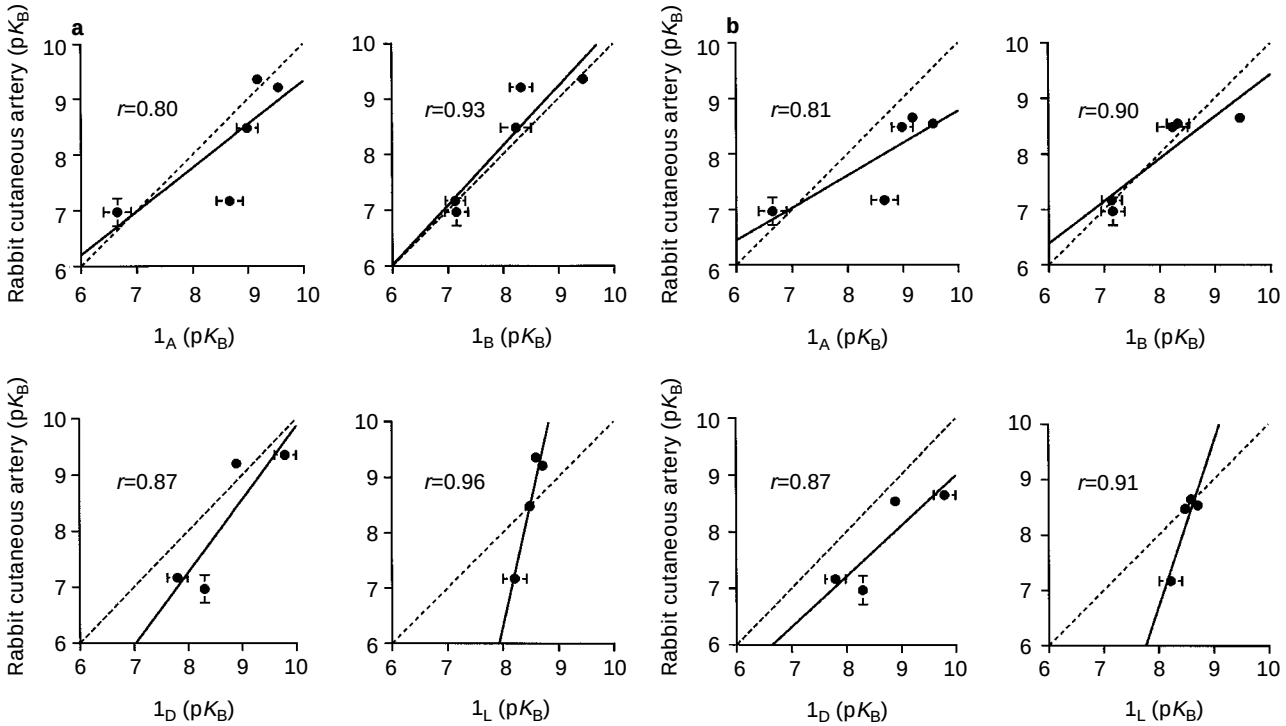

Figure 7 Correlations of potencies of antagonists on rabbit subcutaneous arteries (values from Table 2) with literature examples of various functional α_1-adrenoceptor subtypes. In (a) K_b values are derived from prazosin and WB4101 at low concentration (1 nM) and in (b) at high concentration (100 nM). K_b values for the other antagonists are similar in (a) and (b), i.e. for HV723 and 5-methyl-urapidil the mean of the values for the 3 concentrations employed in Table 2 were used; BMY7378 is used at 1 μM. The comparator tissues have been characterized as containing functional α_{1A}-(rat vas deferens, rat caudal artery), α_{1B}-(rat spleen, human internal iliac artery, dog vertebral and carotid arteries), α_{1D}-(rat aorta) or α_{1L}-(dog femoral artery and femoral vein, rabbit mesenteric artery, thoracic aorta, carotid artery and guinea pig thoracic aorta) adrenoceptors (Aboud *et al*., 1993; Feng *et al*., 1996; Hatano *et al*., 1994; Kenny *et al*., 1995; Kohno *et al*., 1994; Lachnit *et al*., 1997; Muramatsu *et al*., 1990a; Muramatsu *et al*., 1990b). Values are mean $\pm$ s.e.mean and r represents the correlation coefficient. Data was not available in the literature for HV723 versus α_{1D} or BMY7378 versus α_{1L}. The dashed line indicates the line of identity in each correlation.

one being hypothetically α_{1B}, and their responses being additive. We find it difficult to place much weight on this observation but include it since this compound is commonly employed in α_{1B}-adrenoceptor classification.

We have plotted correlations of the ranges of potencies (pK) of prazosin, WB4101, HV723 and 5-methyl-urapidil from rabbit cutaneous resistance arteries against potencies of these same antagonists taken from the literature at functional α_1-adrenoceptor subtypes in tissues which have been characterized, by the authors, as containing α_{1A}-, α_{1B}-, α_{1D}- or α_{1L}-adrenoceptors. By use of pK_B values resulting from low concentrations of prazosin (1 nM) and WB4101 (1 nM), our data produce reasonably linear correlations with the α_{1A}- ($r=0.80$), α_{1B}- ($r=0.93$), α_{1D}- ($r=0.87$) and α_{1L}-subtypes ($r=0.96$). However, the plot resembled the line of equal values most closely for α_{1B}- (Figure 7a). Correlation analysis with the higher concentrations of prazosin (100 nM) and WB4101 (100 nM) also resulted in a good linear correlation with α_{1L}- ($r=0.91$), α_{1D}- ($r=0.87$) and α_{1B}- ($r=0.90$) but a lower correlation with the α_{1A}-subtype ($r=0.81$). In this case the values again lay close to equality against α_{1B}-, with the exception of prazosin (which was lower in rabbit c.r.a.) but also showed equality with α_{1L}-, with the exception of 5-methyl-urapidil (which was lower in rabbit c.r.a.) (Figure 7b). Taken together this tends to reinforce the hypothesis of two functional subtypes of α_1-adrenoceptor in these resistance arteries, or at least to suggest that it is difficult to distinguish between α_{1B}- and α_{1L}-subtypes.

We suggest that the simplest interpretation of our data, on the basis of the current, antagonist-based subclassification of functional α_1-adrenoceptors, is that rabbit cutaneous resistance arteries express a prazosin-sensitive α_1-adrenoceptor subtype, unlikely to be an α_{1D}, most probably the α_{1B}-subtype and, also, an α_{1L}-subtype, both of which are involved in mediating vasoconstriction. However, it should be borne in mind that the agonist data suggest α_{1A}- and that the distinction between α_{1A}- and α_{1B}- is heavily dependent on the low affinity for 5-methyl-urapidil, a compound which has a very variable effect.

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